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# Phytochemical screening and antimicrobial effects of some indigenous medicinal plants on the MDR sewage wastewater bacteria of clinical importance

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## ABSTRACT

Antimicrobial resistance has now become a serious global issue, responsible for hundreds of thousands of deaths worldwide. Treating with traditional synthetic antibiotic is becoming less effective and, in some cases, ineffective. So, there is an urgent need to find alternative natural antimicrobials to combat the MDR (multidrug resistant) bacteria. Medicinal plants contain a variety of primary and secondary metabolites, including carbohydrates, proteins, lipids, alkaloids, glycosides, tannins, phenols, and flavonoids that have been in use through centuries as remedies for infectious diseases. Phytochemical screening and antimicrobial activity of five medicinal plants: *Nerium olender*, *Vitex negundo*, *Commelina benghalensis*, *Calotropis gigantea*, *Eclipta alba* were done against five MDR bacterial strains of sewage canal origin, namely *Weigmannia coagulans*, *Aeromonas caviae*, *Enterobacter ludwigii*, *Bacillus albus* and *Bacillus subtilis* by agar-well diffusion as well as agar dilution methods. *Vitex nedundo* and *Eclipta alba* extracts showed inhibitory effect against *Enterobacter ludwigii* and *Bacillus subtilis*, while *Commelina benghalensis* had sensitivity against *Bacillus subtilis*, and *Catotropis gigantea* inhibit the growth of *Aeromonas caviae*, *Enterobacter ludwigii* and *Bacillus albus*. The *Calotropis gigantean* leaf extract was found excellent to inhibit the growth of *A. caviae*, *B. albus* and *E. ludwigii* with MIC (minimum inhibitory concentration) values 125 – 275 µg/ml, whereas the respective MICs of *Vitex negundo* and *Eclipta alba* were 450 and 400 µg/ml for *Enterobacter ludwigii*, and 150 and 225 µg/ml for *Bacillus subtilis*. The *Commelina benghalensis* leaf extract had strongest antibacterial activity with MIC of of 125 µg/ml. thus, successful isolation and purification of the phytochemicals having antibacterial activity might play important role in the development of alternative natural biotherapeutics of plant origin for combating bacterial infections.

**Keywords:** Antibacterial activity, plant extracts, zone diameter of inhibition, minimum inhibitory concentration, phytochemicals.

## 1. INTRODUCTION

Antimicrobial resistance is now a global issue responsible for more than 7 lakh deaths worldwide, and it has been estimated to kill 350 million people by 2050, according to report by the World Health Organization (WHO). As a consequence of fast antimicrobial resistance, antibiotic treatments are becoming less effective and, in some cases, ineffective. A negative impact exerts on the developing countries in this connection, so there is an urgent need to find alternatives of synthetic antibiotics to combat the infection caused by MDR (multidrug resistant) bacteria (Silver and Bostian, 1993). Medicinal plants contain a variety of primary and secondary metabolites, including carbohydrates, proteins, lipids, alkaloids, glycosides, tannins (Bansal et al., 2021; Chopra et al., 2022). Plant extracts are used to treat various infectious diseases throughout the history of mankind by means of herbal preparations (Wood, 2017; Singh et al., 2020). Products from plants continue to offer mankind novel remedies in various ailments. Secondary metabolites obtained from plants found to have antimicrobial properties (Umashankar, 2020; Lewis and Ausubel, 2006). In this regard, many researchers screened plant extracts to detect secondary metabolites with the relevant antibacterial activities. Antibacterial effects of phytochemicals have documented by Cragg et al. (1997). Several methods have been developed for screening of phytochemicals (Hamburger and Hostettmann, 1991). Antimicrobial activities of *Eclipta alba* (family: Asteraceae) have been tested against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by Sharma et al. (2022). Mamidala (2013) documented antimicrobial activity of petroleum ether, ethyl acetate, ethanol and aqueous extracts of *E. alba* against *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella enterica* serovar Typhi, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis*. The author detected alkaloids, saponins, flavonoids, phenols, tannins, sterols, cardiac glycosides and anthraquinone glycosides in the extracts (Mamidala, 2013). Antibacterial activity of *Calotropis gigantea* (family: Asclepiadaceae) was observed with the extracts of bark and leaves against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Bacillus subtilis* (Mehmood et al., 2020). The plant extract tested was effective against gram-negative (*Porphyromonas gingivalis*) and gram-positive (*Solobacterium moorei*) as reported by Saddiq et al. (2022). *Vitex negundo* (family: Verbenaceae), according to (Mani et al., 2013), contained carbohydrates, proteins, alkaloids, cardiac glycosides, anthraquinones, saponins, flavonoids, and tannins. Methanolic leaf extract of *Vitex negundo* showed inhibitory property against *Bacillus subtilis* and *Streptococcus aureus* (Niranjan et al., 2020). *Nerium olender* (family: Apocynaceae) leaves and flowers contain phenols, tannins, phlobatannins, flavonoids, coumarins, alkaloids, sterols and triterpenoids. Saponin and anthraquinones were reported to be absent in olender (Redha, 2020). Ethanolic extract of *Commelina benghalensis* (family: Commelinaceae) flower showed inhibitory property against *Escherichia coli*, *Staphylococcus aureus*, wherein the extract contained alkaloids, lactones, coumarins, triterpenoids, steroids, tannins, quinones, flavonoids, and saponins (Cuellar et al., 2010).

## 2. MATERIALS AND METHODS

In the current study, a total of five bacterial isolates: *Weigmannia coagulans*, *Aeromonas caviae*, *Enterobacter ludwigii*, *Bacillus albus* and *Bacillus subtilis* were used as the target strains (Das and Mandal, 2022). Bacterial samples were sewage canal in origin and multiple drug resistant (MDR), which are maintained in nutrient agar stab in the laboratory.

Based on their antimicrobial properties, reported by several workers (Wood, 2017; Singh et al., 2020), five plant specimens having ethno-pharmacological importance have been collected from Gurudas College medicinal plants garden, Kolkata, India (22.5713° N, 88.3903° E), the details of which are represented in Table 1. The plant extracts (10%; w/v) of crude powdered plant materials were prepared by using Soxhlet apparatus (Borosil, India) (Das and Mandal, 2022). After extraction, solvents have been condensed by rotary evaporator (Hightech, Germany) at 60°C for 4 h at reduced pressure (-20 hg).

**Table 1:** Plant samples, their families, parts used and extracting solvents.

Plant	Family	Parts collected	Solvent
<i>Vitex negundo</i>	Verbenaceae	Leaves	Ethanol
<i>Eclipta alba</i>	Asteraceae	Whole plant	Ethanol
<i>Nerium olender</i>	Apocynaceae	Leaves	Ethanol
<i>Calotropis gigantea</i>	Asclepiadaceae	Leaves	Ethanol
<i>Commelina benghalensis</i>	Commelinaceae	Whole plants	Methanol

At the concentration of 500 µg/ml of different plant extracts were applied on the test bacteria (10<sup>8</sup> CFU) swabbed on the surface of sterile Mueller-Hinton agar (Hi-Media, India) in Petri plates, to see their antimicrobial activity by agar-well diffusion method

(Mandal et al., 2007; Sircar and Mandal (2016). After incubation for 24 h at 35°C, the assessment of antibacterial activity was done on the basis of measurement of the zone diameter of the inhibition (ZDI) formed around the well, or paper disc.

The MIC (minimum inhibitory concentration) values were determined by agar dilution method (Mandal et al., 2007), using different concentrations of crude plant extracts ranging from µg/ml 25 to 500 µg/ml using Muller-Hinton agar. Agar plates were inoculated with approximately 10<sup>4</sup> CFU/spot, and then incubated at 35°C for 24 h. MIC was considered as the lowest concentration of plant extracts at which no visible growth was found (Mandal et al., 2007).

The qualitative analysis of phytochemicals from plant extracts were done, in order to detect the presence of anthraquinones steroids flavonoids terpenoids tannins alkaloids phenols phytosterols saponin, following the previously published protocols (Kanawa et al., 2019).

### 3. RESULTS AND DISCUSSION

The phytochemicals of the five indigenous plant extracts (*Nerium olender*, *Vitex negundo*, *Commelina benghalensis*, *Calotropis gigantea*, *Eclipta alba*) as determined through quantitative analysis, are represented in Table 2. All the plant extracts were characterized for the presence of diverse groups of phytochemicals, which mainly included tannins and flavonoids. Alkaloids had also been detected in the test plant extracts excluding *Commelina benghalensis*.

The antibacterial activity of the plant extracts, in terms of ZDI as determined by agar-well diffusion, has been depicted in Table 3. As reported by the earlier authors (Sharma et al., 2022), *Eclipta alba* leaves extract showed antibacterial activity against *E. coli* (ZDI: 22 mm), *Klebsiella pneumoniae* (ZDI: 19 mm), *Staphylococcus aureus* (ZDI: 23 mm) and *Pseudomonas aeruginosa* (ZDI: 21 mm). The *Nerium olender* leaf extract inhibited bacterial growth against *Escherichia coli* and *Staphylococcus aureus* displaying ZDIs of 16 mm and 18 mm, respectively (Redha, 2020). Ethanolic extract of *Commelina benghalensis* fresh leaves showed ZDIs of 17 mm and 11 mm against *E. coli* and *Staphylococcus aureus*, respectively (Cuellar et al., 2010). Methanolic leaf extract of *Vitex negundo* had been reported to show antibacterial activity against gram-positive bacteria: *Bacillus subtilis* (ZDI: 13 mm) and *Streptococcus aureus* (ZDI: 13 mm), as reported by (Koirala et al., 2020). In the current study, among the five plant extracts used *Calotropis gigantea* ethanolic leaf extract showed inhibitory effect against three test bacteria (*Aeromonas caviae*, *Enterobacter ludwigii* and *Bacillus albus*). Highest inhibitory activity, with ZDI of 20 mm, was displayed by the methanolic extract of *Commelina benghalensis* leaf against *Bacillus subtilis*. The ethanolic *Nerium olender* leaf extract (ZDI: 6 mm) had no such inhibitory effect against any test bacterial strain. The extracts of *Vitex negundo* and *Eclipta alba* had inhibitory effects against *Enterobacter ludwigii* (ZDI: 16 – 17 mm) and *Bacillus subtilis* (ZDI: 10 – 11 mm). The plant extracts utilized in the current study had no growth inhibitory property against *Weigmannia coagulans*.

**Table 2:** Phyto-components five medicinal plant extracts.

Phytocomponents	NO	VN	CB	CG	EA
Alkaloids	+	+	NT	+	+
Tannins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Glycosides	NT	NT	+	NT	+
Anthraquinones	–	+	–	NT	+
Saponin	–	+	+	+	+
Sterols	+	+	–	+	+
Terpenoids	+	+	–	+	NT
Amino acids	NT	+	NT	NT	NT

NO: *Nerium olender*, VN: *Vitex negundo*, CB: *Commelina benghalensis*, CG: *Calotropis gigantea*, EA: *Eclipta alba*, (+): positive, (–): negative, NT: not tested.

**Table 3:** Antibacterial activity of plant extracts

Plant extract	ZDI (mm) against bacterial isolates				
	<i>W. coagulans</i>	<i>A. caviae</i>	<i>E. ludwigii</i>	<i>B. albus</i>	<i>B. subtilis</i>
<i>V. negundo</i>	6	6	17	6	16
<i>E. alba</i>	6	6	10	6	11
<i>N. oleander</i>	6	6	6	6	6
<i>C. gigantea</i>	6	13	13	11	6

<i>C. benghalensis</i>	6	6	6	6	20
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ZDI: zone diameter of inhibition

**Table 4:** Minimum inhibitory concentration (MIC) values of plant extracts against bacterial isolates

Bacteria	MIC of plant extracts (µg/ml)			
	<i>V. negundo</i>	<i>E. alba</i>	<i>C. benghalensis</i>	<i>C. gigantea</i>
<i>W. coagulans</i>	>500	>500	>500	>500
<i>A. caviae</i>	>500	>500	>500	125
<i>E. ludwigii</i>	450	150	>500	275
<i>B. albus</i>	>500	>500	>500	150
<i>B. subtilis</i>	400	225	125	>500

The MICs of the plant extracts, as determined by agar-well diffusion, has been represented in Table 4. The *Calotropis gigantea* leaf extract was found excellent to inhibit the growth of *A. caviae*, *B. albus* and *E. ludwigii* with MICs 125 – 275 µg/ml. The extracts of *V. negundo*, *E. alba* and *C. benghalensis* had MICs 400, 225 and 125 µg/ml against *B. subtilis*, while *E. ludwigii* was inhibited by *C. gigantea*, *V. negundo* and *E. alba* with MICs 150 – 275 µg/ml. As has been reported by (Gurrapu and Mamidala, 2017), *Eclipta alba* leaf alkaloids had MICs of 42 – 89 µg/ml against *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella boydii* *Staphylococcus aureus* and *Streptococcus faecalis*. *Eclipta alba* leaf alkaloids showed antibacterial activity displaying ZDI values 9 – 17 mm (with 125 – 500 µg/ml extracts), against different gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella boydii*) and gram-positive (*Staphylococcus aureus* and *Streptococcus faecalis*).

Ethanol and ethyl acetate extracts of *Eclipta prostrata* leaves of the plant have been found to be active against *E. coli*, *K. pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* with MICs ranging from 4.5 to 90 µl/ml (Karthikumar et al., 2007). MIC of *Vitex negundo* methanolic leaf extract against *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi* ranged 0.612 – 5 mg/ml. (Kumar et al.2013). The crude aqueous extract of *Calotropis gigantea* (Apocynaceae) latex displayed antibacterial activity against gram-positive (*Staphylococcus aureus*, *Bacillus cereus* and *Micrococcus luteus*) and gram-negative (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) bacteria with MICs 62.5 – 125 µg/ml (Kumar et al., 2010), while the leaf extract MICs for the bacterial isolates ranged 12.5 – 50 mg/ml (Kumar et al., 2010a). The various extracts of *Commelina benghalensis* had MICs 8 – 128 µg/ml against gram-positive (*Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Staphylococcus pyogenes* and *Streptococcus agalactiae*) as well as gram-negative (*Salmonella typhi*, *Escherichia coli*, *Shigella boydii*, *Shigella dysenteriae* and *Pseudomonas aeruginosa*) bacteria (Khan et al., 2011).

#### 4. CONCLUSION

Among five medicinal plants studied, four namely *Vitex negundo*, *Commelina benghalensis*, *Calotropis gigantea*, *Eclipta alba* showed excellent antibacterial activity against one or more bacterial strains tested, following agar dilution as well as disc diffusion methods. This antibacterial capacity of the plants was attributed because of the presence of various phytochemicals. Therefore, such plants might be utilized in the preparation of plant-based treatment against bacterial infection.

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This study has not received any external funding.

#### Conflicts of interests

The authors declare that there are no conflicts of interests.

#### Data and materials availability

All data associated with this study are present in the paper.

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